The University of Chicago

THE PHYSIOLOGICAL ACTIVITY OF IODINE IN THYROGLOBULIN

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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BRODA OTTO BARNES

From the Department of Physiology, University of Chicago

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Attempts to prepare a characteristic substance from the thyroid gland began certainly as early as 1884 when Bubnow (1) prepared "Thyreoprotein." Langerdorff (2) made some observations on the colloid of the thyroid and noticed that it became readily soluble when subjected to artificial gastric digestion. Gourley (3) prepared a substance which he called the "principal proteid" of the thyroid. From the properties of his compound, it must have been the same as Bubnow's preparation. It remained for Baumann (4) to discover iodine in the gland in 1895. His correlation of the iodine content with diseases of the thyroid not only paved the way for iodine therapy but also gave a chemical means of following the active principle in thyroid preparations. Attempts to concentrate or isolate iodine-containing compounds characteristic of thyroid action may be classified under the three following heads: acid hydrolysis, alkaline hydrolysis, and by the use of enzymes. Only the latter will be considered here since it is closer related to experimental hyperthyroidism produced by feeding thyroid preparations.

Most of the work involving enzyme hydrolysis is open to one of two criticisms: either very little was done in attempting to separate the iodine compound or else the fractions were not properly assayed. Hutchison (5) as early as 1896 reported that the protein which Bubnow and Gourley had described contained iodine. He carried out a series of experiments on this compound including the solubility, color reactions, heat of coagulation, hydrolysis with acid, and the effect of gastric digestion. By the latter method, he separated the iodine into two fractions which were then assayed on thyroidectomized dogs in tetany. His conclusions are unwarranted in view of the present conception of tetany and the parathyroids. The same author (6) later reported the separation of digested thyroid into three fractions: iodothyrin, albumoses and peptones. The iodine content decreased in the order mentioned. They were tested on a case of myxedema and their effect on loss of weight was proportional to their iodine content. Tamback (7) studied the effect of both pepsin and trypsin on

thyroid preparations, but did not study their physiological activity. Some digestion studies on human thyroids were carried out by von Cyon and Oswald (8), but their products were tested on the circulation and not on thyroid deficient animals. Oswald (9) in 1908 reported quantitative studies on thyroglobulin digested with trypsin. His report that as much as two-thirds of the iodine might be liberated in inorganic form has probably kept many workers from using enzymes. However, it must be remembered that the active principle is not destroyed, at least entirely, when thyroid is fed. Furthermore, Oswald did not examine his mixtures until months after their preparation. The length of time and possible bacterial contamination might have added to the decomposition. Nurnberg (10) carried out some extensive chemical studies on the products after digestion of thyroglobulin, but he did not test them physiologically. Pick and Pineles (11) were the first to combine both separation and adequate assay. Using thyroidectomized goats which displayed cretin symptoms, they found that fresh thyroids, thyroglobulin, and secondary albumoses relieved the thyroid deficiency. On the other hand, iodothyrin, primary albumoses, and digestive mixtures standing three months were ineffective. Cameron and Carmichael (12) made the first attempts to compare the effects of undigested thyroglobulin with the products of digestion. Although they state that their method of assay, using body weight and hypertrophy of the kidneys, liver and heart, is not quantitative, the digested mixture had the same order of activity as the original protein. They examined the residue after digestion, the filtrate, and, in one case, the peptones prepared from the filtrate. All of these fractions were active by their assay. Last year Harington and Salter (13) employed enzymes for the isolation of thyroxine from thyroglobulin. They discarded the filtrate at pH 5.0, apparently without testing its potency. This is not surprising, since Kendall (14) had reported that the acid-soluble fraction after alkaline hydrolysis was inactive. Harington and Randall (15) had confirmed this and shown that thyroxine was quantitatively precipitated at pH 5.0.

Numerous reports have been made that, in equivalent amounts of iodine, thyroxine is less active than desiccated glands. On the other hand, it seems definitely agreed that only a portion of the iodine in the thyroid is present as thyroxine. Therefore one must postulate either that the active principle is more potent than thyroxine per se or that other compounds are present in desiccated thyroid which add to the effects of thyroxine. A study was undertaken to test various fractions of artifically digested thyroglobulin. Since it was found that the filtrate at pH 5.0, after tryptic digestion, might contain as much as fifty per cent or more of the total iodine, this filtrate was examined in some detail for its physiological actions.

METHODS. 1. *Preparation of thyroglobulin digest.*[1] The thyroglobulin used was prepared by a method to be described later. The principle involved the precipitation of the protein from thyroid extract at the isoelectric point. A portion of the thyroid extract was reserved in order to compare the effects of the original active principle with it after digestion. The precipitated thyroglobulin was put into one per cent sodium bicarbonate, and commercial pancreatin was added to a concentration of 0.2 per cent. A few drops of alcoholic thymol were added as a preservative. Although the bicarbonate tends to keep the reaction of such a mixture slightly alkaline, the pH was adjusted daily to 8.0. The pancreatin was renewed after 48 hours. After being in the incubator for 72 hours, the mixture was brought to a pH of 5.0, and the precipitate was collected on a filter. This procedure differs from Harington's only in the preparation of thyroglobulin and in the presence of the bicarbonate. A control solution was prepared by digesting casein in a similar manner. However, this digest was not fractionated.

2. *Work on rats.* Since the digested filtrate might contain toxic substances, it was administered orally by a stomach tube. This was accomplished under light ether anesthesia. The animals were placed on their backs and a small tube attached to a syringe was gently pushed down through the esophagus into the stomach. The fluid could then be injected without any trouble or danger of loss. The dosage varied from 2 to 4 cc. depending on the size of the animal. The procedure was so simple that the rat was under the anesthetic only 2 or 3 minutes.

3. *Work on rabbits.* Two rabbits were given the filtrate by means of a stomach tube, and two others were given the dried precipitate put into capsules. No anesthesia was necessary. When the capsules were given, the animals were observed closely until they had swallowed all of the material. The body weight and rectal temperature were recorded daily.

4. *Work on dogs.* Three dogs were employed for basal metabolism studies. They were trained to lie quietly, and the calories per 24 hours were determined using the Benedict portable apparatus. After control observations had been made (at least 10), the filtrate was given to two of the dogs by stomach tube. Five doses were administered and the heat production, heart rate, respiration and rectal temperature determined. At the end of this experiment, one of these dogs and the third one were fed the precipitate after digestion of the thyroglobulin. The quantities of both the precipitate and the filtrate employed contained the same amount of iodine.

RESULTS. In a preliminary experiment, two preparations of the filtrate at pH 5.0 were prepared and tested for thyroid activity by the loss of

[1] I am indebted to Armour & Company for the fresh thyroids.

weight in rats. Four animals were given each preparation, and four animals were anesthetized in the same way and given a similar quantity of tap water as a control. Of the 8 experimental animals, all lost weight at the beginning but recovered somewhat at the end of 3 weeks. The weight losses amounted to as much as 20 grams in several cases, and one animal died with a loss of 28 grams. At the end of three weeks only one animal was heavier than at the beginning, while the controls had gained weight consistently. These experiments indicated that there was some thyroid-like activity in the filtrate. It was then decided to use more rats, rabbits, and basal metabolism tests on dogs in order to investigate this indication thoroughly.

Experiments on rats. To examine further the effects of ether on growth and the body weight, forty white rats ranging from 70 to 130 grams in weight were divided into two groups. Half of these were anesthetized daily for a period of 7 days. Neither group showed any weight loss during the week, and at the end of the experiment the controls had gained an average of 15.7 grams while those anesthetized averaged 13 grams. If ether did retard growth, certainly it was to a minor degree. More animals would have to be employed to be sure that there was any significant effect. A few days intervened before the same animals were divided into three groups, two of ten each and one of twenty. One group of ten received the original thyroid extract, the other small group received the same volume of casein digest. The large group was given the same volume of digested thyroglobulin filtrate, which contained the same quantity of iodine as the original thyroid extract. The dosage was regulated so that it contained an amount of iodine equivalent to two grams of U. S. P. desiccated thyroid per kilo body weight. The average daily variation for each group is recorded in figure 1. It can be seen that all three groups lost weight at the beginning, but the effect was much greater with the thyroid extract. No explanation can be given for the loss of weight in the animals on casein, where growth was retarded, since at the end of the week they had gained only 4.7 grams. According to the experiment where only ether was given, the gain should have been 13 grams. Since this weight loss has been obtained once with casein and three times with thyroglobulin fractions which contain very little if any thyroxine, one is forced to raise the question whether toxic substances which cause loss of weight are produced by artificial digestion. No attempt will be made to answer this question at present, but the loss of weight in rats has been abandoned as a method of thyroid assay.

The group of animals on the filtrate from digested thyroglobulin lost a little more weight than those on casein, and at the end of the week were only back to their original weight. One cannot interpret this as an indication of thyroid activity in view of what has just been said. However,

their weight loss was in no way comparable with that of the animals on thyroid extract. Here the weight loss was much more severe and five of the animals had died within four days. On the seventh day the dosage of both the original extract and the digested filtrate was increased. Three days later only two of the ten rats on the extract were alive and only one from the group of 20 had died. This latter animal had lost no weight and probably died from other causes. These results clearly show that the digested filtrate contains very little thyroid activity in comparison to the original extract, when equal quantities of iodine are administered.

Experiments on rabbits. The dosage in terms of iodine for the rabbits receiving the filtrate was equivalent to 2 grams of desiccated thyroid per kilo. Unfortunately one of these animals was killed accidentally early

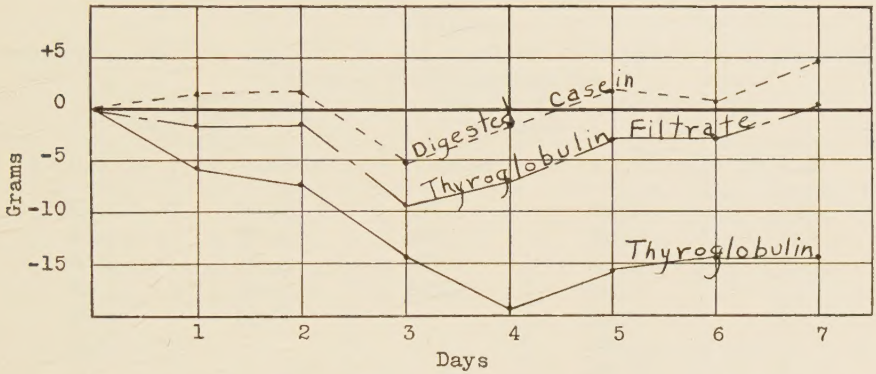

Fig. 1. Showing the average daily changes in weight of rats fed digested casein, undigested thyroglobulin, and the filtrate obtained by bringing digested thyroglobulin to pH 5.0.

in the experiment. The data are summarized in table 1. The rabbit receiving the filtrate continually lost weight during the ten-day period. Although in this case a total of over 400 grams was lost, there was never any diarrhea observed. After the experiment stopped the animal started to regain weight, but observations were not continued until recovery was complete. This experiment furnishes further evidence that there is a factor causing loss of weight, and in this instance there was no anesthesia. There seems a significant difference, however, between the rats and the rabbits. The rats make some adjustment to the toxic factor while the rabbits do not, at least within ten days.

The two rabbits fed the precipitate from thyroglobulin digest exhibited quite a different reaction. Their weight loss was so severe that only three doses were given. The dosage in this case was only three-fourths as great, or the equivalent of one and one-half grams of desiccated thyroid

per kilo. Both animals had severe diarrhea from the second day until they died. When their weights had dropped over 400 and 300 grams respectively in 3 days, the feeding was stopped in order to allow recovery. However, the weather was hot at this time and the animals did not survive the toxicity, one dying the following day and the other 24 hours later. This rapid loss of weight would lead one to believe that the precipitate was very active physiologically, but no quantitative comparisons can be made.

No change in the temperature of the animal receiving the filtrate was ever noticed. Daily fluctuations with changes in the weather were the same as in the control animals. The same may be said for animal 4. The day before animal 3 died his temperature was 2°C. higher than at any other time. In summarizing the data on rabbits, it would appear

TABLE 1

The daily weight of rabbits fed the filtrate and the precipitate obtained by bringing digested thyroglobulin to pH 5.0

DAYS	FILTRATE		PRECIPITATE	
	No. 1	No. 2	No. 3	No. 4
0	2,420	1,740	1,960	1,960
1	2,360	1,700	1,900	1,930
2	2,320	1,620	1,680	1,740
3	2,240	Killed	1,540	1,620
4	2,200		Dead	1,560
5	2,140			Dead
6	2,160			
7	2,120			
8	2,060			
9	2,000			
10	1,960			

that there is some factor in the filtrate which causes loss of weight but does not cause diarrhea or elevation of body temperature. The precipitate causes a much more rapid loss of weight, severe diarrhea, and may elevate body temperature.

Experiments on basal metabolism. Both the filtrate and precipitate were tested for their effect on basal metabolism, since it is probably the final test for thyroid activity. Large doses which were equivalent in iodine to 13 grams of desiccated thyroid were employed. Five daily doses were given to dogs 1 and 2. It can be seen in table 2 that the filtrate had no effect on basal metabolism. This was a surprise since the weight loss in both rats and rabbits had indicated some activity. Just how little hormone is necessary to raise basal metabolism cannot be stated, but from the work of Kunde (16) it would appear that there was not much, if any,

of the active principle present. There was no change in heart rate, temperature, or respiration that would indicate any hyperthyroidism.

When the precipitate having an amount of iodine equivalent to that in the filtrate was fed the basal metabolisms showed a significant rise within 24 hours. Dog 2 showed a gradual rise until on the third day the heat production was over 31 per cent above the controls. Only three doses were fed after which the basal metabolic rate came down gradually. On the seventh day, or 4 days after the last feeding of the thyroid preparation, the basal metabolism was within the control range. Dog 3 did not reach the high peak until the fourth day although none of the material

TABLE 2

The daily changes in basal metabolism and heart rate produced by feeding the filtrate and precipitate obtained by bringing digested thyroglobulin to pH 5.0

DAYS	DOG 1		DOG 2		DOG 3		REMARKS
	Calories per 24 hours	Heart rate	Calories per 24 hours	Heart rate	Calories per 24 hours	Heart rate	
0	341.3	102	330.5	61	439.0	54	Average of controls
1	335.1	105	334.2	62			Given 100 cc. filtrate
2			325.6	60			Given 100 cc. filtrate
3	344.7	93	329.6	60			Given 100 cc. filtrate
4	340.6	80	326.6	62			Given 100 cc. filtrate
5	322.3	78	353.2	60			
0							1.1 gm. pt. fed
1			366.8	70	483.7	74	1.1 gm. pt. fed
2			387.9	84	479.9	64	1.1 gm. pt. fed
3			433.7	84	512.6	73	Fed no thyroid
4			411.3	74	600.5	79	Fed no thyroid
5			389.9	68	504.0	63	Fed no thyroid
6			356.2	62	492.0	58	Fed no thyroid
7			347.2	58			

was fed on the previous date. The return to normal was also slower than the other dog. The heart rate paralleled the basal metabolism in both cases and was surprisingly close in dog 2, whose normal rate was quite constant and never varied over 4 beats per minute in the control period. The heart rate in each case returned to normal before the heat production. In dog 2 the temperature was elevated 0.5°C. on the third, fourth, and fifth days. Dog 3 showed a rise of 0.2 degree only on the fourth day, when his basal metabolism was also at its peak. Respiration did not parallel the heat production as closely as the heart beats, but the respiratory rate on the day of maximum basal metabolism was double the rate at any other time.

Although these observations indicate that the precipitate has considerable potency, no quantitative comparisons can be made. It is apparent that the iodine of the thyroglobulin can be split into two definite fractions by the use of pancreatin. Since one of these fractions shows comparatively little, if any, activity, it would seem conclusive proof that all of the iodine is not associated with the active principle. This agrees with the evidence of Kendall and Harington that all of the iodine is not present as thyroxine. However, the evidence that desiccated thyroid is more active per iodine content than thyroxine has not been satisfactorily explained. Further studies on the precipitate will be made in order to see if some of the active principle might have been destroyed by the artificial digestion.

SUMMARY

1. By the use of pancreatin, thyroglobulin was split into an acid-soluble fraction and an acid-insoluble fraction. The iodine content of each fraction was about the same in some cases. The acid-soluble fraction caused a loss of weight in rats and rabbits. It does not cause elevation of body temperature, increase in basal metabolism or diarrhea.

2. The acid-insoluble fraction caused much greater weight losses, severe diarrhea, elevation of body temperature, increased heart rate, rapid respiration, and a rise in basal metabolism.

3. If artificial digestion has not destroyed any of the physiological activity, at least only half of the total iodine in the thyroid is combined with the active principle.

I wish to take this opportunity to thank Mr. G. F. Stewart for making the pH adjustments for me. It has been a great opportunity and a pleasure to carry out this work under the direction of Dr. A. J. Carlson, whose advice and encouragement have meant so much.

BIBLIOGRAPHY

(1) Bubnow. Zeitschr. f. physiol. Chem., 1884, viii, 1.
(2) Langendorff. Quoted from Hutchison.
(3) Gourlay. Journ. Physiol., 1894, xvi, 23.
(4) Baumann. Zeitschr. f. physiol. Chemie, 1895, xxi, 319.
(5) Hutchison. Journ. Physiol., 1896, xx, 474.
(6) Hutchison. Journ. Physiol., 1898–99, xxiii, 178.
(7) Tamback. Zeitschr. Biol., 1898, xxxvi, 549.
(8) von Cyon and Oswald. Pflüger's Arch., 1901, lxxxiii, 199.
(9) Oswald. Arch. f. exper. Path. u. Pharm., 1908, lx, 115.
(10) Nürnberg. Biochem. Zeitschr., 1909, xvi, 87.
(11) Pick and Pineles. Zeitschr. exper. Path., 1909–10, vii, 518.
(12) Cameron and Carmichael. Trans. Roy. Soc. Canada, 1926, xx, 307.
(13) Harington and Salter. Biochem. Journ., 1930, xxiv, 456.
(14) Kendall. Journ. Biol. Chem., 1915, xx, 501.
(15) Harington and Randall. Biochem. Journ., 1929, xxiii, 373.
(16) Kunde. This Journal, 1927, lxxxii, 195.